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CASE REPORT

Labile Hemoglobin - A Biochemical Entity

Rinu Goyal [1], *Vandana Sardana* [1*]

[1] Sitaram Bhartia Institute of Science and Research, New Delhi, India

ABSTRACT

Diabetes mellitus is a major health disease and may vary in clinical presentation and progression. Although Glucose Fasting and Post prandial levels give a measure of the current plasma glucose level, Glycated haemoglobin is the key analyte used to assess the glycemic control and risk of complications of patients with diabetes. Labile hemoglobin A1c is also known as pre-HbA1c or LA1c or pre-glycohemoglobin. It is an unstable form, (a Schiff base) formed during non-enzymatic glycation of hemoglobin. The concentration of labile fraction varies with acute change in plasma glucose level. Labile hemoglobin may potentially interfere in the estimation of HbA1c and cause a falsely low value and may even hamper or delay the prompt treatment required in diabetics. This calls for a detailed and careful study of chromatograms generated for each sample.

Keywords: glycated hemoglobin, labile hemoglobin, acute changes in plasma glucose, chromatogram

Correspondence:

Vandana Sardana

Address: Sitaram Bhartia Institute of Science and Research, New Delhi, India

Email: vandana2121@gmail.com

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INTRODUCTION

Glycated haemoglobin was initially identified as the 'unusual haemoglobin' in diabetic patients around 40 years ago. It is now the cornerstone in diabetic clinics. HbA1c is the haemoglobin that is covalently bound to glucose. It reflects the average plasma glucose over the last 8 – 12 weeks. HbA1c is the preferred investigation to assess the glycemic control of a patient specially because it gives a good measure of the risks of diabetic complications - the higher the measured HbA1c, the more is the risk of complications associated with diabetes. Also, it is a very convenient test because no special patient preparation is required. During the formation of Glycated Hemoglobin, an unstable Schiff base is obtained. Also called Labile Hemoglobin, it marks acute changes in the blood glucose level.

MATERIALS AND METHODS

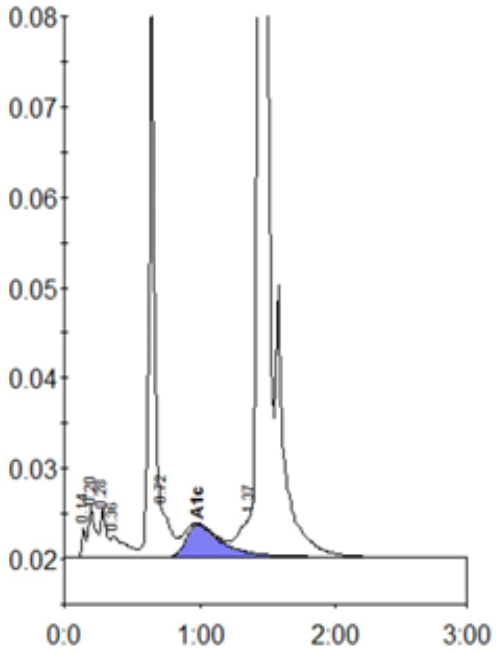
This article presents a case report at Sitaram Bhartia Institute of Science and Research, New Delhi, a non profit health organisation. In the hospital's in-house laboratory, HbA1c is processed using High Performance Liquid Chromatography (HPLC) on the Biorad D-10 analytical system. Patient samples are run after obtaining a successful pass report of two levels of internal quality control- Biorad Lyphocheck Diabetes Control - level 1& level 2.

OBSERVATIONS

On 5/6/19, the laboratory received a patient sample from the OPD of Sitaram Bhartia Institute of Science and Research. Upon running the sample, we obtained a chromatogram which showed LA1c/CHb-1 with an area of 8.3% and LA1c/ CHb-2 with an area of 2.4% (a total of 10.7%) and HbA1c with an area of 4.4%. Seeing a low value of HbA1c, we enquired the patient history. We came to know that the patient was a diabetic and she had received treatment for high plasma glucose levels four days back. Knowing that acute changes in the plasma glucose level could cause a high Labile haemoglobin value, we advised the patient to come after a gap of one day. On 7/6/19, the result of the repeat sample tested showed LA1c/CHb-1 window with an area of only 1.9% and HbA1c with an area of 7.5%. The chromatograms of samples run on 5/6/19 and 7/6/19 are shown below:

Patient report

Bio-Rad	DATE: 07/17/2019
D-10	TIME: 05:07 PM
S/N: #DJ7A055307	Software version: 4.30-2
Sample ID:	D369463
Injection date	06/05/2019 02:45 PM
Injection #: 12	Method: HbA1c
Rack #: ---	Rack position: 4



Peak table - ID: D369463

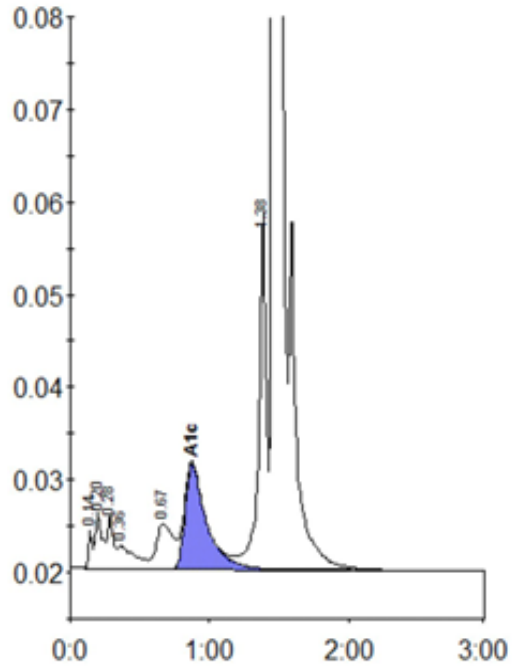
Peak	R.time	Height	Area	Area %
Unknown	0.14	3458	6968	0.3
A1a	0.20	5955	19896	0.9
A1b	0.28	5537	15731	0.7
Unknown	0.36	2301	18919	0.9
LA1c/CHb-1	0.64	71666	180171	8.3
LA1c/CHb-2	0.72	4548	52666	2.4
A1c	0.98	3608	66328	4.4
P3	1.37	4559	32797	1.5
A0	1.44	526117	1783123	81.9
Total Area:			2176599	

Concentration:	%
A1c	4.4

Figure 1. First sample taken on 05.06.2019

Patient report

Bio-Rad	DATE: 07/17/2019
D-10	TIME: 05:08 PM
S/N: #DJ7A055307	Software version: 4.30-2
Sample ID:	BHARTI
Injection date	06/07/2019 02:12 PM
Injection #: 9	Method: HbA1c
Rack #: ---	Rack position: 1



Peak table - ID: BHARTI

Peak	R.time	Height	Area	Area %
Unknown	0.14	4281	8618	0.4
A1a	0.20	6164	21285	1.0
A1b	0.28	5786	16761	0.8
Unknown	0.36	2479	20858	1.0
LA1c/CHb-1	0.67	4819	39802	1.8
A1c	0.87	11407	122153	7.5
P3	1.38	38653	139261	6.4
A0	1.47	577321	1818814	83.1
Total Area:			2187551	

Concentration:	%
A1c	7.5

Figure 2. Second sample taken on 07.06.2019 (after 1 day)

DISCUSSION

Glycated haemoglobin assesses the average plasma glucose over a period of 2-3 months. When haemoglobin is exposed to glucose, the carbonyl group of glucose binds to the N-terminal of the valine residue of beta chain of Adult haemoglobin (HbA). A reversible reaction takes place and forms an Unstable Schiff's base, called Labile Hemoglobin. This represents acute changes in the plasma glucose levels. During RBC circulation, the unstable Hemoglobin undergoes Amadori rearrangement and gets converted into a stable Ketoamine called Glycated Hemoglobin or HbA1c. Glycated haemoglobin is a widely used and a preferred investigation in all diabetic clinics specially because it gives quite an accurate measure of the risk of microvascular and macrovascular complications associated with Diabetes like retinopathy, nephropathy and neuropathy and also because it does not demand any special patient preparation, like a fasting state.

Glycated Hemoglobin can be estimated by the following methods, namely Ion Exchange High Performance Liquid Chromatography, Immunoassay, Enzymatic Assay, Boronate Affinity High Performance Liquid Chromatography and Capillary Electrophoresis. Ion Exchange High Performance Liquid Chromatography, commonly called HPLC, separates the analytes chromatographically based on ion exchange. The separated analytes are measured spectroscopically at 415nm and a chromatogram is generated. HbA1c is calculated using an Exponentially Modified (EMG) algorithm, the advantage of which is that there is a separate peak obtained for Labile and Carbamylated Hemoglobin. Advantages of High Performance Liquid Chromatography over other methods are: a short testing time, cost effectiveness, Labile A1c and Carbamylated peak areas being excluded from A1c peak, an alert for Variant window, a good repeatability result, besides being a sensitive and automated method.

Some factors may cause falsely low values, like conditions which decrease the life span of RBC (haemolytic anemia, acute early stages blood loss), Chronic liver disease,, Vit C and E (inhibit glycation), Pregnancy (associated with lower fasting blood glucose level), drugs used to treat malignancy, HIV, Hepatitis C virus, chronic ingestion of Salicylates, Alcoholism and Lipidemia. Factors causing a falsely high values of A1c include Vit B12 and Folate deficiency (increased RBC life span), Iron Deficiency Anemia (Increased RBC turnover, increased glycation), and Splenectomy. Factors interfering with glycated haemoglobin are serum hyperbilirubinemia, hypertriglyceridemia, HbF > 10%, Labile A1c > 4% and CHb > 3.5%.

Labile haemoglobin is the unstable form, a Schiff base, so is a measure of the acute changes of the plasma glucose level. Labile hemoglobin should be noted carefully while estimating HbA1c by HPLC technique as it is a potential source of pre-analytical error. Each chromatogram generated needs to be studied by pathologist/biochemist as well as clinician. This will reduce any chance of misinterpretation of report and inappropriate clinical decision while managing the patient.

Some key points to be kept in mind while reporting the chromatogram: 1. Total area of the graph must be between 1 million and 5 million. For samples with an area more than 5 million, the sample should be diluted and rerun. 2. Quality control values should be in range. 3. A1c and A0 peaks should be distinct and should be correctly identified with separate retention times. 4. HbF peak area should be less than 10% 5. Labile A1c (LA1c/CHb-1) should be less than or equal to 4%. 6. Carbamylated hemoglobin (LA1c/CHb2) should be less than or equal to 3.5%. 7. HbA1c should be in the reportable range. 8. The baseline should be properly constructed, straight and not be drifting. 9. A1c peak obtained should be sharp and symmetrical.

SUMMARY AND CONCLUSION

Labile Hemoglobin is an Unstable Schiff base and is eluted in the ion exchange chromatographic method (HPLC). A value of >4% LA1c represents any acute changes in the plasma glucose and could be an important source of pre-analytical error. High Performance Liquid Chromatography is the method of choice for performing Glycated Hemoglobin because it is not only cost effective and involves shorter testing time, this method also gives a separate window for Labile haemoglobin and an alert for the presence of variant window. It is of utmost importance to read and study the chromatogram generated for each sample with great care so that such cases, with acute changes in plasma glucose levels, are not missed on HbA1c test because HbA1c is the parameter which gives reliable information about the risk of complications associated in patients with Diabetes.

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